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CORRELATION BETWEEN ANTI-INFLAMMATORY PROPERTIES AND INHIBITION OF PROSTAGLANDIN BIOSYNTHESIS *IN VITRO*

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Abstract—Four chemically related compounds (two benzofurans, one thianaphthene and one indolyl derivative) have been compared with aspirin and indomethacin in their ability to inhibit prostaglandin (PG) synthesis *in vitro* by microsomes of bull seminal vesicles with tritiated arachidonate as substrate. Two of these substances [L 8027: 3-(2-isopropyl indolyl)-3 pyridyl ketone and L 8109: (5-chloro-3-methyl-2-benzo [b] thienyl) acetic acid] inhibit the synthesis and show marked anti-inflammatory action in the usual pharmacological tests; however, there is no strict correlation, the most powerful inhibitor being the least active anti-inflammatory agent. A benzofuran derivative with a low anti-inflammatory action, but distinctly fibrinolytic [L 2197: 2-ethyl-3-(4-hydroxybenzoyl) benzofuran] does not inhibit PG synthesis. A powerful fibrinolytic agent (L 7035: 2-isopropyl-3-isonicotinoyl benzofuran), practically devoid of anti-inflammatory action, is a more active inhibitor of PG synthesis than aspirin. It seems that the PG synthetase system of the seminal vesicles does not react *in vitro* to inhibitors in the same way as that responsible for PG synthesis in tissues damaged by carrageenan or the Freund adjuvant. Such a discrepancy might be due to differences in the tissue distribution of the inhibitors and this could explain the lack of correlation between the *in vivo* and the *in vitro* potencies of drugs like L 8027 and L 8109. Localization of L 7035, on the other hand, would be such that the anti-aggregating properties can fully develop, while the inflamed sites remain out of reach of the drug.

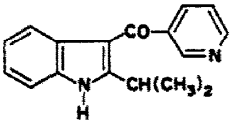
The prostaglandins play an important rôle in inflammatory reactions. Numerous nonsteroidal anti-inflammatory agents, of which aspirin is the prototype, inhibit the biosynthesis of prostaglandins in many organs, platelets and mammalian cells in culture [1, 2]. Enzyme preparations from ram or bull seminal vesicles have frequently been used to analyze this inhibition. We have investigated the effect on prostaglandin biosynthesis of four compounds chosen from a recently synthetized series in order to see if there is any correlation between inhibition of this biosynthesis and anti-inflammatory action revealed by the usual techniques of experimental pharmacology‡.

MATERIAL AND METHODS

Compounds. We have chosen from a large series four chemically related compounds (Fig. 1) and compared their action with that of aspirin and indomethacin. The reasons for this choice are the following: (a) L 8027 and L 8109 meet all the experimental criteria of anti-inflammatory substances [4, 5], L 8109 is an acid, L 8027 is basic; (b) L 7035 is a powerful fibrinolytic agent but does not decrease, even at high doses, the paw oedema after injection of carrageenan or *Mycobacterium* extracts (Freund arthritis); (c) L 2197 has practically no anti-inflammatory action, its fibrinolytic action is weaker than that of L 7035 [6, 7].

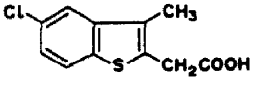
Biochemical techniques. The compounds were dissolved in propylene glycol. Bull seminal vesicles, kept in dry ice, were carefully dissected; fatty and connective tissues were discarded. The vesicles were cut in small fragments, 12 g of which were homogenized at 4° in 25 ml of 0·15 M Sörensen phosphate buffer pH 8. The homogenate was centrifuged in the cold at 4,000 *g* for 10 min; the supernatant was centrifuged at

L 8027: 3-(2-isopropyl indolyl) 3-pyridyl ketone



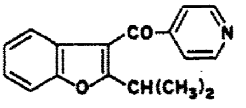
(antiinflammatory, antiarthritic)

L 8109: (5-chloro 3-methyl 2-benzo [b] thienyl) acetic acid



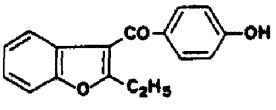
(antiarthritic, antiinflammatory)

L 7035: 2-isopropyl 3-isonicotinoyl benzofuran



(fibrinolytic, thrombolytic)

L 2197: 2-ethyl 3-(4-hydroxybenzoyl) benzofuran



(fibrinolytic, thrombolytic)

Fig. 1.

‡ A preliminary note on L 8027 was published in 1973 [3].

Table 1. Biosynthesis of PGE by the microsomial fraction of bull seminal vesicle in the presence of various substances

Drug	Concentration (M)										
	3×10^{-3}	10^{-3}	3×10^{-4}	10^{-4}	5×10^{-5}	3×10^{-5}	10^{-5}	5×10^{-6}	3×10^{-6}	10^{-6}	10^{-7}
L 8027		10·9 ± 0·4 (10)	20·0 ± 0·9 (8)	24·1 ± 0·7 (8)			47·6 ± 1·7 (10)		62·0 ± 2·4 (6)	69·2 ± 2·4 (8)	97·4 ± 4·2 (8)
L 7035		23·1 ± 1·0 (10)	51·1 ± 2·2 (8)	52·2 ± 1·6 (10)		72·9 ± 4·5 (6)	84·6 ± 3·2 (7)	97·6 ± 4·1 (10)			
L 8109	32·7 ± 1·8 (8)	44·2 ± 1·3 (10)	66·5 ± 2·7 (8)	78·7 ± 2·4 (9)	99·5 ± 3·1 (10)						
L 2197		93·0 ± 2·4 (16)		98·9 ± 2·9 (17)							
Acetylsalicylic acid	22·7 ± 1·3 (8)	33·0 ± 1·1 (8)	58·3 ± 3·6 (6)	61·5 ± 2·2 (8)			94·1 ± 4·9 (8)				
Indomethacin		12·2 ± 0·7 (6)		37·5 ± 1·5 (8)			56·4 ± 2·4 (8)			78·8 ± 5·0 (6)	

Results are expressed in per cent of controls ± S.E.M. Values in brackets indicate number of assays.

Table 2.

	I_{50} of the prostaglandin synthetase	Anti-inflammatory actions in the rat AD_{50} (mg/kg) by oral route				
	(1)	(2)	(3)	(4)	(5)	(6)
Aspirin	$3{\cdot}3 \times 10^{-4}$	100	100	50	250	500
Indomethacin	$2{\cdot}1 \times 10^{-5}$	4	4	0·2	0·3	1·5
L 8027	$1{\cdot}0 \times 10^{-5}$	22	13	3	5	50
L 8109	$8{\cdot}3 \times 10^{-4}$	22	13	0·5	0·7	2·5
L 7035	$1{\cdot}7 \times 10^{-4}$	100 to 300	—	300	100 to 300	—
L 2197	(7)	100	100 to 200	100	300	400.

(1) Molar concentrations inducing a 50 per cent inhibition of the prostaglandin synthetase of bull seminal vesicles microsomes (I_{50}) calculated by least-squares regression analysis from the values of Table 1.
(2) Carrageenan oedema.
(3) U.v. erythema.
(4) *Mycobacterium butyricum* injected in the hind leg (results on paw of injected leg).
(5) *Mycobacterium butyricum* injected in the hind leg (results on paw of controlateral leg).
(6) *Mycobacterium butyricum* injected in the tail.
(7) Inactive (See Table 1).

100,000 *g* for 30 min. The pellet, consisting mainly of microsomes, was suspended in 25 ml of 0·2 M Tris–HCl, pH 8·0.

The substrate solution contained 100 μg glutathione, 0·7 μg hydroquinone, 150 μg arachidonic acid (all-*cis* eicosa-5,8,11,14-tetraenoic acid) (Schwartz–Mann), 5 μCi arachidonic acid $CH_3(CH_2)_4(C^3H{=}C^3HCH_2)_4COOH$ (sp. act. 18·2 Ci/m-mole) (New England Nuclear), 50 ml 0·2 M tris–HCl, pH 8·0. The tritiated arachidonic acid was purified by chromatography before each series of assays, using chloroform–methanol–acetic acid–water (90:6·5:1:0·75, by vol.) as solvent. The suspension of microsomes (0·5 ml) and the substrate solution (2 ml) were preincubated separately at 37° for 15 min. After mixing, the compound to be tested, in 0·1 ml of propylene glycol, was added and the incubation continued for 25 min at 37° in a Dubnov shaker. The reaction was stopped by addition of 0·25 ml 2 M citric acid. Control incubations confirmed that the amount of labelled prostaglandins (>95% PGE_2) increased rapidly during the first twelve minutes and remained constant thereafter. We chose a 25 min incubation time to ensure that the reaction, when inhibited, had reached a plateau. This method, used by many scientists interested in *in vitro* biosynthesis of prostaglandins, is the best available at the present time because the system is polyenzymatic and that technically speaking the rapidity of reaction prevents an accurate estimation of the initial slope.

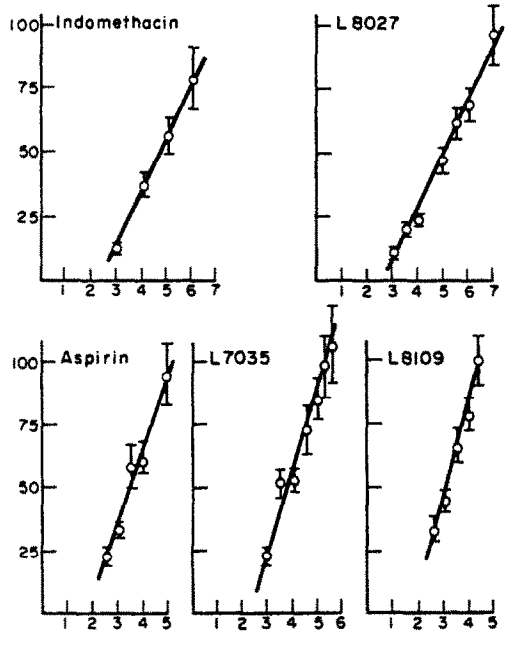


Fig. 2. Action of drugs on enzymatic activity. Ordinate: enzyme activity (A) in per cent of control. Abscissae: −log molar drug concentration. Lines represent the best fit, as determined by linear least-squares regression analysis. Vertical bars represent standard deviations.

After addition of 50 μg unlabelled PGE_2, the system was extracted three times with 10 ml of diethylether; the organic fractions were evaporated to dryness, dissolved and subjected to thin layer chromatography on aluminium sheet coated with Merck silicagel using chloroform–methanol–acetic acid–water (90:6:1:0·75, by vol.) as solvent or on glass plate coated with Merck silicagel using benzene–dioxane–acetic acid (40:40:2, by vol.) as solvent. These operations and the localization of the active spots have been described in a previous paper [8]. The PG spots were scraped off, eluted with ether–methanol (9:1, v/v) and collected directly in scintillation vials. The residue after evaporation was dissolved in Instagel® or Bray's fluid and radioactivity was measured in a Packard Tricarb scintillation counter. Controls, acidified to pH 3 by addition of citric acid, gave the necessary blank values. Normal activity of the microsome suspension was measured in ten controls.

Results are expressed in per cent of the activity of these controls after subtraction of the basal activity.

RESULTS AND DISCUSSION

Tables 1 and 2 and Fig. 2 summarize our results; Table 2 compares the anti-inflammatory activity of the four compounds tested with that of aspirin and indomethacin. It is clear that for this limited series of substances there is no correlation between anti-inflammatory activity and inhibition of prostaglandin synthesis

by the bull seminal vesicle enzyme system. L 8109 is as active as L 8027 in the carrageenan oedema and u.v. erythema tests and much more active in the *Mycobacterium* test, but more than 100 times less active on PG synthesis. L 2197, a fibrinolytic agent, shows no inhibitory action on prostaglandin synthesis although its anti-inflammatory effects are comparable with that of aspirin. L 7035, which is a powerful fibrinolytic agent, is about as active as aspirin on the prostaglandin synthetase, but is practically devoid of anti-inflammatory action. This is particularly interesting since Gryglewski in 1970 (i.e. just before the discovery of the inhibition of prostaglandin synthesis by nonsteroidal anti-inflammatory agents) attempted in a very stimulating review [9] to correlate fibrinolytic with anti-inflammatory action.

More information is needed, however, about the action of these substances on prostaglandin synthesis by microsomes from organs other than bull seminal vesicle. The case of paracetamol which inhibits prostaglandin synthesis in brain and not in other tissues is probably not unique. The PG synthetase of platelets, smooth muscle and epithelia of the blood vessels may have different sensitivity to L 7035.

Which are the systems important for the local or general inflammatory reactions to carrageenan or *Mycobacterium* extracts? The seminal vesicles which offer nice possibilities for biochemical studies are not important but the blood vessels may be the structures mainly involved in increased prostaglandin synthesis during inflammation.

Several authors have shown that the injection of moderate amounts of sodium arachidonate into rats or rabbits results in a decrease of arterial pressure. Since this hypotensive effect is abolished by indomethacin [10, 11], it is logically argued that PG synthesis is responsible for this effect. In collaboration with G.Barac, two of us (C.D. and Z.M.B.) have recently shown that arachidonate-induced hypotension is potentiated by injection of heparin and tryptophan in dogs as well as in rabbits [12, 13]. In rabbits, L 8027 (4 mg/kg) and L 8109 (4 mg/kg) completely abolished the hypotension following arachidonate injection, but L 7035 (4 mg/kg) did not reduce the fall in blood pressure. Thus in this case there seems to be a correlation between the arachidonate test and the anti-inflammatory action. In our opinion, there is no question that the general theory developed by Vane, Collier, Flower and associates, is of great value.

The apparent lack of correlation between the anti-inflammatory potencies of L 8027 and L 8109 and their inhibitory action on the PG synthetase system might result from differences in their distribution patterns in the organism. Likewise, L 7035 might fail against inflammation and arachidonic acid-induced hypotension because of its peculiar distribution (the drug is firmly bound to proteins). In this case, however, other sites of PG synthetase probably come into play, since the drug [7] is a potent fibrinolytic and anti-aggregating agent. Hence, L 7035 would be a case in which pharmacokinetics and tissue affinity might explain apparent discrepancies to the theory of Vane.

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